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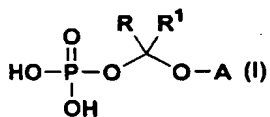
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(54) Title: WATER SOLUBLE PRODRUGS OF AZOLE COMPOUNDS



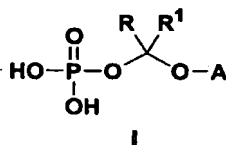
(57) Abstract: Water-soluble prodrugs of triazole antifungal compounds having a secondary or tertiary hydroxy group are provided. More particularly, new water-soluble triazole antifungal compounds are provided having the general formula (I) wherein A is the non-hydroxy portion of a triazole antifungal compound of the type containing a secondary or tertiary hydroxyl group and R and R¹ are as defined in the specification.

WATER SOLUBLE PRODRUGS OF AZOLE COMPOUNDS

5

BACKGROUND OF THE INVENTION

This invention relates to novel water-soluble azole compounds useful for the treatment of serious systemic fungal infections and suitable for both oral and, particularly, parenteral administration. More particularly, the invention relates to
10 novel water-soluble prodrugs having the general formula:



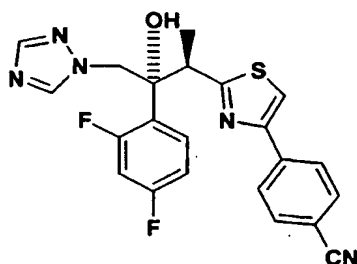
wherein A is the non-hydroxy portion of a triazole antifungal compound of the
15 type containing a secondary or tertiary hydroxy group, R and R¹ are each independently hydrogen or (C₁-C₆)alkyl, or pharmaceutically acceptable salts thereof.

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DESCRIPTION OF THE PRIOR ART

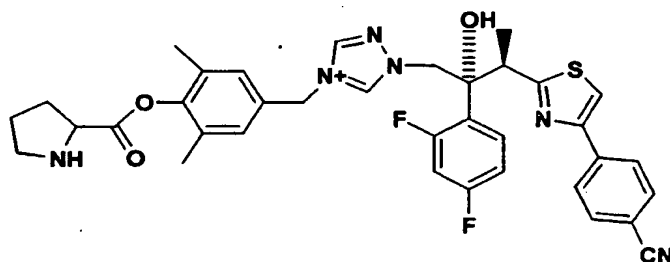
Triazole antifungal compounds are well known in the prior art. Of the several classes known, one particularly potent class contains a tertiary hydroxyl group. For example, U. S. Patent 5,648,372 discloses that (2R,3R)-3-[4-(4-cyanophenyl)thiazol-2-yl]-2-(2,4-difluorophenyl)-1-(1H-1,2,4-triazol-1-yl)-
25 butan-2-ol has anti-fungal activity.

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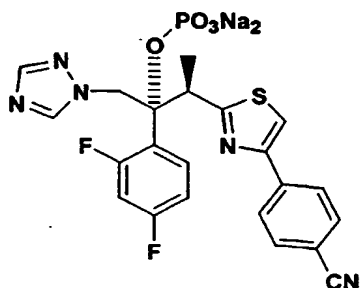


The utility of this class of compounds is limited by their low water solubility. For example, the solubility of the above triazole compound in water at pH 6.8 is 0.0006 mg/mL. This greatly impedes developing suitable parenteral dosage forms.

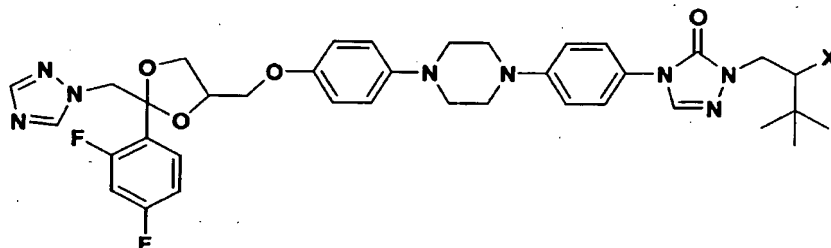
One method of addressing this problem was disclosed in European Patent Application 829478, where the water solubility of an azole antifungal agent was increased by attaching a linked amino-acid to the azole portion of the molecule



Alternatively, WO 97/28169 discloses that a phosphate moiety can be attached directly to the tertiary hydroxyl portion of the anti-fungal compound, e.g. the compound having the formula

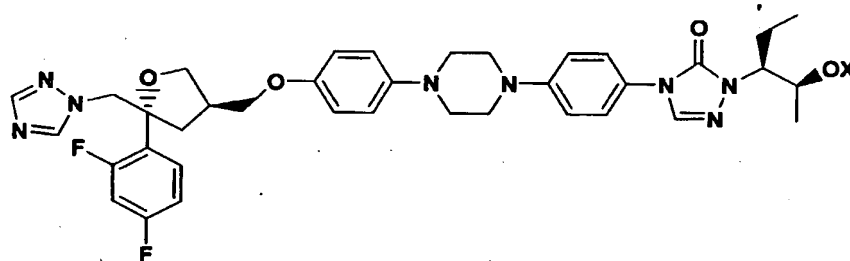


U.S. Patent 5,707,977 and WO 95/19983 disclose water soluble prodrugs having the general formula



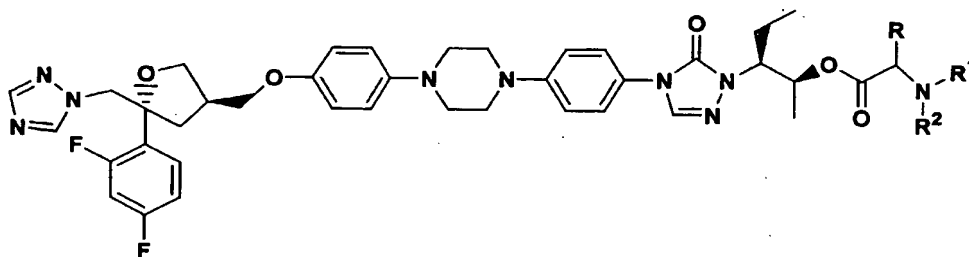
wherein X is OP(O)(OH)_2 or an easily hydrolyzable ester $\text{OC(O)RNR}^1\text{R}^2$.

WO 95/17407 discloses water-soluble azole prodrugs of the general formula

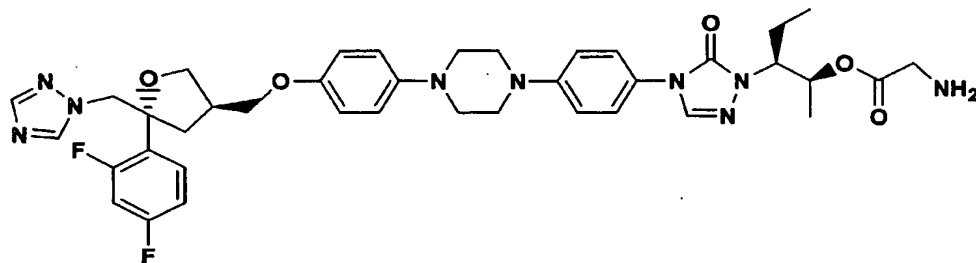


wherein X is $P(O)(OH)_2$, $C(O)-(CHR^1)_n-OP(O)(OH)_2$ or $C(O)-(CHR^1)_n-(OCHR^1CHR^1)_mOR_2$.

WO 96/38443 discloses water-soluble azole prodrugs of the general formula



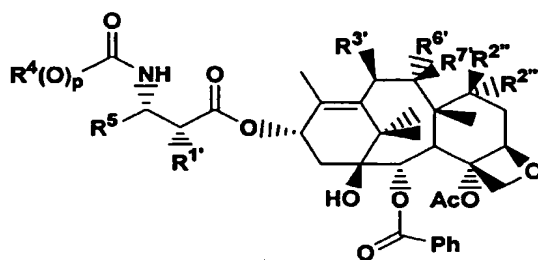
U.S. Patent 5,883,097 discloses water-soluble amino acid azole prodrugs such as the glycine ester



The introduction of the phosphonooxymethyl moiety into hydroxyl
5 containing drugs has been disclosed as a method to prepare water-soluble prodrugs of hydroxyl containing drugs.

European Patent Application 604910 discloses phosphonooxymethyl
taxane derivatives of the general formula

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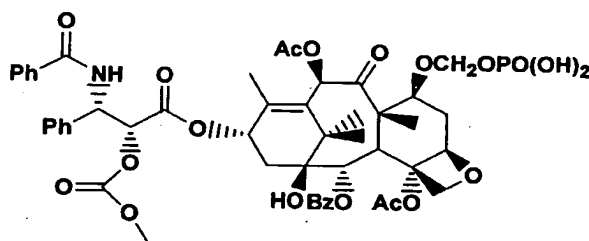


wherein at least one of $R^{1'}$, $R^{2''}$, $R^{3'}$, $R^{6'}$ or $R^{7'}$ is $OCH_2OP(O)(OH)_2$.

European Patent Application 639577 discloses phosphonooxymethyl
15 taxane derivatives of the formula $T-[OCH_2(OCH_2)_mOP(O)(OH)_2]_n$ wherein T is a taxane moiety bearing on the C13 carbon atom a substituted 3-amino-2-hydroxypropanoyloxy group; n is 1, 2 or 3; m is 0 or an integer from 1 to 6 inclusive, and pharmaceutically acceptable salts thereof.

WO 99/38873 discloses O-phosphonooxymethyl ether prodrugs of a diaryl 1,3,4-oxadiazolone potassium channel opener.

Golik, J. et al, Bioorganic & Medicinal Chemistry Letters, 1996, 6:1837-1842 discloses novel water soluble prodrugs of paclitaxel such as



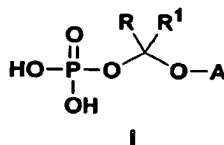
SUMMARY OF THE INVENTION

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It has now been found that triazole anti-fungal compounds containing a secondary or tertiary hydroxyl group, including (2R,3R)-3-[4-(4-cyanophenyl)thiazol-2-yl]-2-(2,4-difluorophenyl)-1-(1H-1,2,4-triazol-1-yl)-butan-2-ol, may be converted into prodrugs with superior properties to those previously disclosed by attaching a phosphate containing moiety via a linking group.

Specifically, the invention covers compounds of the formula:

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20 wherein A is the non-hydroxy portion of a triazole antifungal compound of the type containing a secondary or tertiary hydroxy group, R and R¹ are each independently hydrogen or (C₁-C₆) alkyl, or pharmaceutically acceptable salts thereof.

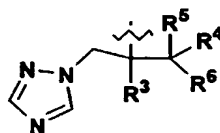
The compounds of general formula I function as "prodrugs" when administered *in vivo*, being converted to the biologically active parent azole in the presence of alkaline phosphatase.

- 5 Preferred among the compounds of formula I are those wherein R and R¹ are both hydrogen.

In a preferred embodiment, A represents the non-hydroxy portion of a triazole antifungal compound of the type containing a tertiary hydroxy group.

10

In a more preferred embodiment of the above type compounds, A can be



wherein R³ represents phenyl substituted by one or more (preferably 1-3) halogen atoms;

15

R⁴ represents H or CH₃;

R⁵ represents H, or taken together with R⁴ may represent =CH₂;

20

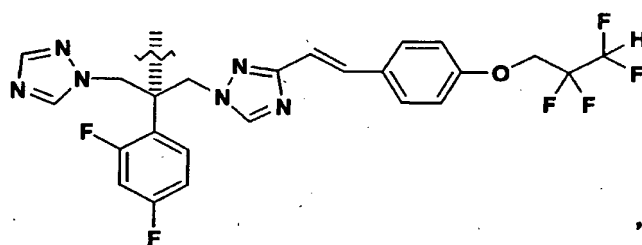
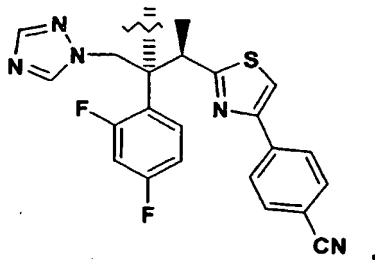
R⁶ represents a 5- or 6 membered nitrogen containing ring which may be optionally substituted by one or more groups selected from halogen, =O, phenyl substituted by one or more groups selected from CN, (C₆H₄)-OCH₂CF₂CHF₂ and CH=CH-(C₆H₄)-OCH₂CF₂CHF₂, or phenyl substituted by one or more groups selected from halogen and methylpyrazolyl.

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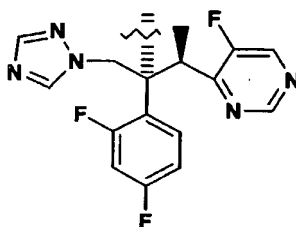
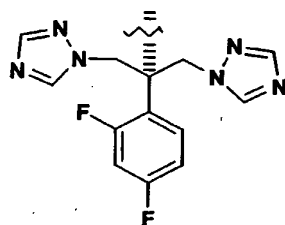
Nitrogen containing heterocycles which R⁶ may represent include triazolyl, pyrimidinyl, and thiazolyl.

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Specific examples of A include, but are not limited to, the following:

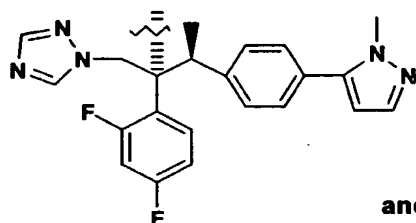
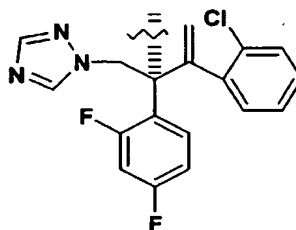


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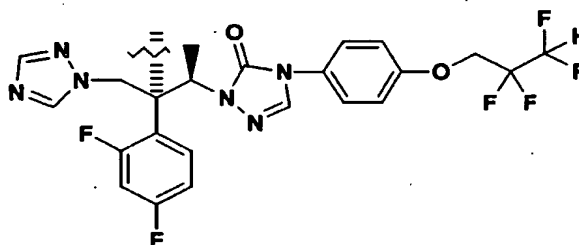


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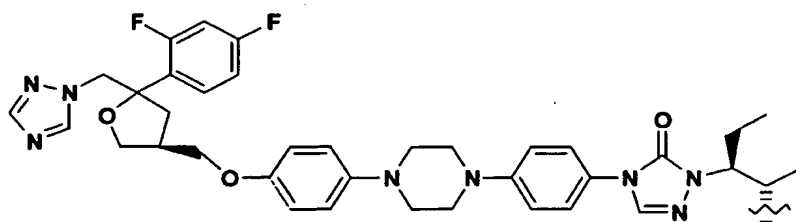
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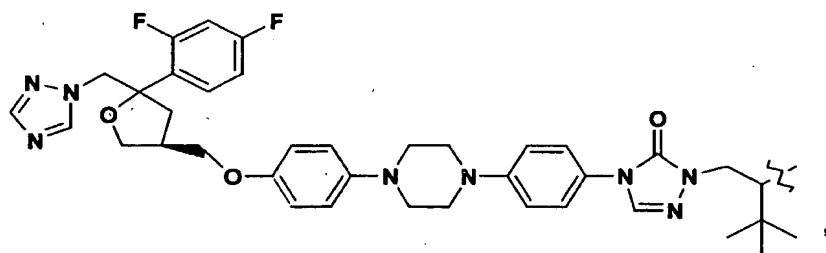


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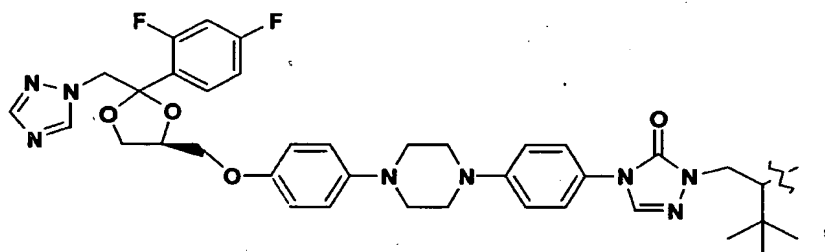
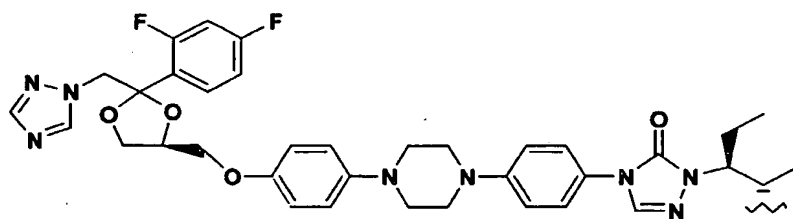
In addition to the application of the present invention to structures
 10 containing a tertiary alcohol, it should also be understood that this discovery can
 be applied to anti-fungal agents which contain secondary alcohols. Some
 examples of the non-hydroxy portion of triazole antifungal compounds of the type
 containing a secondary hydroxy group include, but are not limited to, the
 following:

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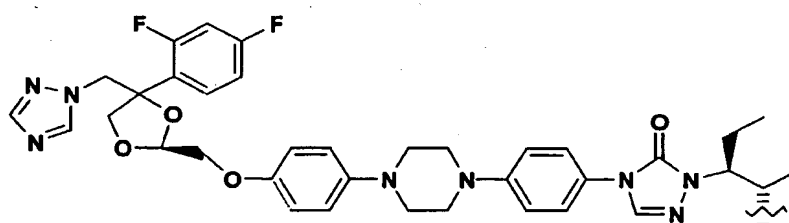




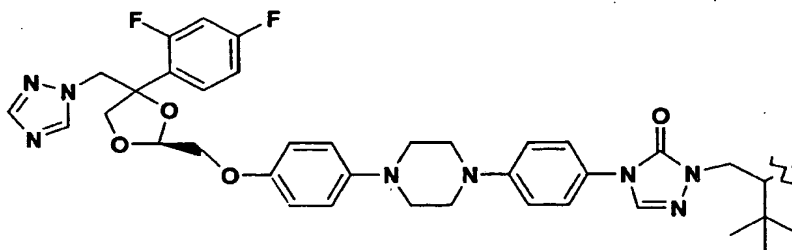
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10



or



DETAILED DESCRIPTION

As used herein "(C₁-C₆)alkyl" refers to a straight or branched chain
 5 saturated aliphatic group having 1 to 6 carbon atoms such as methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, t-butyl, n-pentyl, n-hexyl, etc.

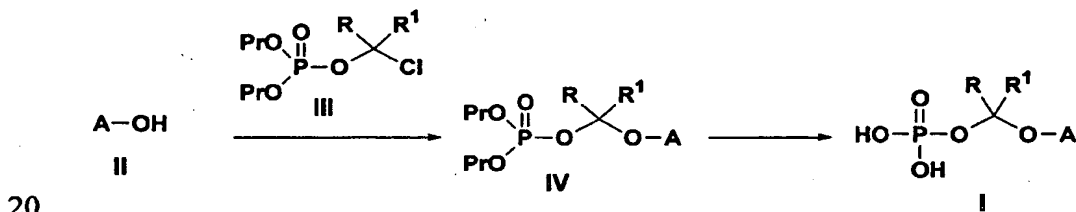
The term "pharmaceutically acceptable salt" as used herein is intended to include phosphate salts with such counterions as ammonium, metal salts, salts
 10 with amino acids, salts with amines and salts with other bases such as piperidine or morpholine. Both mono- and bis-salts are intended to be encompassed by the term "pharmaceutically acceptable salts". Specific embodiments include ammonium, sodium, calcium, magnesium, cesium, lithium, potassium, barium, zinc, aluminum, lysine, arginine, histidine, methylamine, ethylamine, t-
 15 butylamine, cyclohexylamine, N-methylglucamine, ethylenediamine, glycine, procaine, benzathene, diethanolamine, triethanolamine, piperidine and morpholine. For the most preferred embodiment, (2R,3R)-3-[4-(4-cyanophenyl)thiazol-2-yl]-2-(2,4-difluorophenyl)-1-(1H-1,2,4-triazol-1-yl)-2-
 20 [(dihydrogen phosphonoxy)methoxy]butane, the t-butylamine and lysine salts are especially preferred as they can be obtained as single polymorph crystalline solids of high purity with good solubility and stability.

The term "halogen" as used herein includes chloro, bromo, fluoro and iodo, and is preferably chloro or fluoro, and most preferably fluoro.

The compounds of the present invention can be solvated or non-solvated. A preferred solvate is a hydrate.

A most preferred embodiment of the present invention is (2R,3R)-3-[4-(4-cyanophenyl)thiazol-2-yl]-2-(2,4-difluorophenyl)-1-(1H-1,2,4-triazol-1-yl)-2-
 5 [(dihydrogen phosphonoxy)methoxy]butane or a pharmaceutically acceptable salt thereof. This prodrug exhibits much improved aqueous solubility (>10 mg/mL at pH 7, 5-6 mg/mL at pH 4.3) compared with the parent compound which enables it to be used for parenteral administration as well as oral administration. This
 10 compound is also stable in solution, can be isolated in crystalline form and is readily converted to parent drug *in vivo*.

The compounds of the present invention may be made by the following general reaction scheme. In this method, A represents the non-hydroxy portion of
 15 a triazole antifungal compound of the type containing a tertiary or secondary hydroxyl group, Pr represents a conventional hydroxy-protecting groups such as t-butyl, benzyl or allyl, and R and R¹ are each independently hydrogen or (C₁-C₆)alkyl. Most preferably, R and R¹ are both hydrogen.



To elaborate on the method, the antifungal parent compound of interest, II, is converted into the phosphate intermediate IV by O-alkylation with chloride intermediate III in the presence of a suitable base such as sodium hydride, potassium hydride, sodium amide, sodium t-butoxide, potassium t-butoxide,
 25 sodium bis(trimethylsilyl)amide, potassium bis(trimethylsilyl)amide, or combinations thereof such as sodium hydride plus sodium bis(trimethylsilyl)amide. This reaction step may be carried out in an inert organic solvent such as tetrahydrofuran, methyl-tetrahydrofuran, methyl t-butyl ether,

diethylether or dimethylacetamide at a temperature of from about 0 ° to 50 °C, more preferably between about 20 ° to 40 °C, and most preferably at about 40° C. The most preferred base is sodium hydride and the most preferred solvent is tetrahydrofuran. The most preferred R and R¹ groups are hydrogen.

5

Ester intermediate IV is then subjected to a conventional deprotection step to remove the hydroxyl-protecting groups Pr. The reagents used in such step will depend on the particular hydroxyl-protecting group used, but will be well known to those skilled in the art. The most preferred hydroxy protecting group is the t-butyl group which can be removed with trifluoroacetic acid, hydrochloric acid or formic acid in an appropriate inert organic solvent. The inert solvent may be, for example, methylene chloride, dichloroethane, methylbenzene or trifluoromethyl benzene. In the case of the preferred deprotection step with the di-tertiary butyl ester, it is preferred to do the deprotection step in trifluoroacetic acid in methylene chloride at a temperature of from about 0 ° to 40 °C, most preferably at a temperature of about 0-5 °C.

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The final product I may then be recovered and purified by conventional procedures such as reverse phase C-18 column chromatography or solvent extraction.

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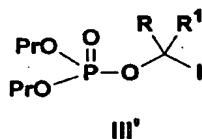
End product I may, of course, be converted by conventional means to a desired pharmaceutically acceptable salt as described above.

25

It was later discovered that use of purified reagent III gave fairly low yields of intermediate IV (approximately 10-35% yield) in the above reaction, resulting in low overall yields of product I. However, when a source of iodide ion is added to the O-alkylation step of the above reaction, the yield of intermediate IV is unexpectedly increased to up to about 90%, thus also significantly increasing the yield of final product I. It is believed that the addition of the iodide

30

ion may result in *in situ* formation of the corresponding iodide intermediate III' of the formula



5 and that use of this reagent results in a large increase in yield of the phosphate intermediate IV. The attempt to substitute preformed intermediate III' directly for intermediate III in the first step of the above reaction, however, was unsuccessful due to the greatly decreased stability of iodide reagent III' compared to the
10 chloride intermediate III. An alternative method which was successful involves using iodine in the O-alkylation step along with chloride intermediate III in the presence of base such as NaH (which also may act as a reducing agent for the iodine). It is believed that the iodine is reduced to iodide ion which then converts
15 chloride intermediate III *in situ* to iodide intermediate III' to facilitate this step of the process. The illustrative example below shows the O-alkylation step using elemental iodine which is the preferred method of carrying out this reaction to get intermediate IV.

20 By forming the iodide reagent III' *in situ* by addition of a source of iodide ion or by reaction of iodine and reagent III in the presence of strong base, the greatly increased yield of phosphate ester IV allows the final product I to be also obtained in greatly increased yield.

25 The source of iodide ion is preferably sodium iodide, but may also include lithium iodide, cesium iodide, cadmium iodide, cobalt iodide, copper iodide, rubidium iodide, barium iodide, zinc iodide and calcium iodide. About 2-3 equivalents of the iodide salt is generally used per equivalent of parent compound A-OH.

When elemental iodine is used in the coupling step, about 0.1 to 1.0 equivalent of iodine, preferably 0.5 equivalent, is employed per equivalent of parent compound A-OH.

- 5 The bases and solvents which are used when iodine or iodide ion is used are the same as those described above when reagent III is used *per se*.

It will be understood that where the substituent groups used in the above reactions contain certain reaction sensitive functional groups such as amino or
10 carboxylate groups which might result in undesirable side-reactions, such groups may be protected by conventional protecting groups known to those skilled in the art. Suitable protecting groups and methods for their removal are illustrated, for example, in Protective Groups in Organic Synthesis, Theodora W. Greene (John Wiley & Sons, 1991). It is intended that such "protected" intermediates and end-
15 products are included within the scope of the present disclosure and claims.

It will be appreciated that certain products within the scope of formula I may have substituent groups which can result in formation of optical isomers. It is intended that the present invention include within its scope all such optical
20 isomers as well as epimeric mixtures thereof, i.e. R- or S- or racemic forms.

The pharmaceutically active compounds of this invention may be used alone or formulated as pharmaceutical compositions comprising, in addition to the active triazole ingredient, a pharmaceutically acceptable carrier, adjuvant or
25 diluent. The compounds may be administered by a variety of means, for example, orally, topically or parenterally (intravenous or intramuscular injection). The pharmaceutical compositions may be in solid form such as capsules, tablets, powders, etc. or in liquid form such as solutions, suspensions or emulsions. Compositions for injection may be prepared in unit dose form in ampules or in
30 multidose containers and may contain additives such as suspending, stabilizing and dispersing agents. The compositions may be in ready-to-use form or in

powder form for reconstitution at the time of delivery with a suitable vehicle such as sterile water.

Alternatively, the compounds of the present invention can be administered
5 in the form of a suppository or pessary, or they may be applied topically in the form of a lotion, solution, or cream. Additionally, they may be incorporated (at a concentration up to 10%) into an ointment consisting of a white wax or soft, white paraffin base together with the required stabilizers and/or preservatives.

10 The compounds of the invention are useful because they possess pharmacological activities in animals, including particularly mammals and most particularly, humans. Specifically, the compounds of the present invention are useful for the treatment or prevention of topical fungal infections, including those caused by species of *Candida*, *Trichophyton*, *Microsporum*, or *Epidermophyton*.
15 Additionally, they are useful for the treatment of mucosal infections caused by *Candida albicans*. They can also be used in the treatment of systemic fungal infections caused, for example, by species of *Candida albicans*, *Cryptococcus neoformans*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Coccidioides*, *Paracoccidioides*, *Histoplasma*, or *Blastomyces*.

20 Thus, according to another aspect of the invention, there is provided a method of treating a fungal infection which comprises administering a therapeutically effective amount of the compound to a host, particularly a mammalian host and most particularly a human patient. The use of the
25 compounds of the present invention as pharmaceuticals and the use of the compounds of the invention in the manufacture of a medicament for the treatment of fungal infections are also provided.

The dosage to be administered depends, to a large extent, on the particular
30 compound being used, the particular composition formulated, the route of administration, the nature and condition of the host and the particular situs and organism being treated. Selection of the particular preferred dosage and route of

application, then, is left to the discretion of the physician or veterinarian. In general, however, the compounds may be administered parenterally or orally to mammalian hosts in an amount of from about 5 mg/day to about 1.0 g/day. These doses are exemplary of the average case, and there can be individual instances
5 where higher or lower dosages are merited, and such dosages are within the scope of this invention. Furthermore, administration of the compounds of the present inventions can be conducted in either single or divided doses.

The *in vitro* evaluation of the antifungal activities of the compounds of the
10 invention can be performed by determining the minimum inhibitory concentration (MIC). The MIC is the concentration of test compound which inhibits the growth of the test microorganism. In practice, a series of agar plates, each having the test compound incorporated at a specific concentration, is inoculated with a fungal strain and each plate is then incubated for 48 h at 37 °C. The plates are examined
15 for the presence or absence of fungal growth, and the relevant concentration is noted. Microorganisms which can be used in the test include *Candida albicans*, *Asperigillus fumigatus*, *Trichophyton spp.*, *Microsporum spp.*, *Epidermophyton floccosum*, *Coccidioides immitis*, and *Torulopsos galbrata*. It should be recognized that, as prodrugs, some compounds of the invention may not be active
20 in the *in vitro* test.

The *in vivo* evaluation of compounds of the present invention can be carried out at a series of dose levels by intraperitoneal or intravenous injection or by oral administration to mice which have been inoculated with a strain of fungus
25 (e.g. *Candida albicans*). Activity is determined by comparing the survival of the treated group of mice at different dosage levels after the death of an untreated group of mice. The dose level at which the test compound provides 50% protection against the lethal effect of the infection is noted.

30 The compounds of the present invention substantially increase the solubility of the parent triazole antifungal compound and also release the

bioactive parent compound (i.e. function as a prodrug) as demonstrated in human liver S9 experiments.

ILLUSTRATIVE EXAMPLES

5

The following examples illustrate the invention, but are not intended as a limitation thereof. The abbreviations used in the examples are conventional abbreviations well-known to those skilled in the art. Some of the abbreviations used are as follows:

10

h	=	hour(s)
rt	=	room temperature
mmol	=	mmole(s)
g	=	gram(s)
15 THF	=	tetrahydrofuran
mL	=	milliliter(s)
L	=	liter(s)
Et ₂ O	=	diethyl ether
EtOAc	=	ethyl acetate
20 TFA	=	trifluoroacetic acid
CH ₂ Cl ₂	=	dichloromethane
CH ₃ CN	=	acetonitrile

In the following examples, all temperatures are given in degrees
25 Centigrade. Melting points were determined on an electrothermal apparatus and are not corrected. Proton nuclear magnetic resonance (¹H NMR) spectra were recorded on a Bruker -500, Bruker AM-300 or a Varian Gemini 300 spectrometer. All spectra were determined in CDCl₃ or D₂O unless otherwise indicated. Chemical shifts are reported in δ units (ppm) relative to
30 tetramethylsilane (TMS) or a reference solvent peak and interproton coupling constants are reported in Hertz (Hz). Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad peak; dd, doublet

of doublets; dt, doublet of triplets; and app d, apparent doublet, etc. Mass spectra were recorded on a Kratos MS-50 or a Finnegan 4500 instrument utilizing direct chemical ionization (DCI, isobutene), fast atom bombardment (FAB), or electron ion spray (ESI).

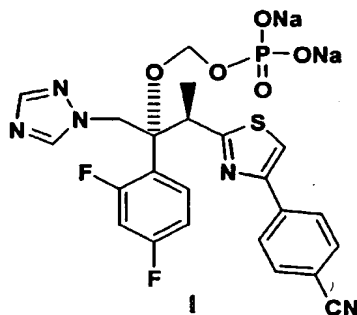
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Analytical thin-layer chromatography (TLC) was carried out on precoated silica gel plates (60F-254) and visualized using UV light, iodine vapors, and/or staining by heating with methanolic phosphomolybdic acid. Reverse phase chromatography was performed in a glass column using C18 silica gel (Waters Corporation Preparative C18 125A) at pressures somewhat above atmospheric pressure.

10

EXAMPLE 1

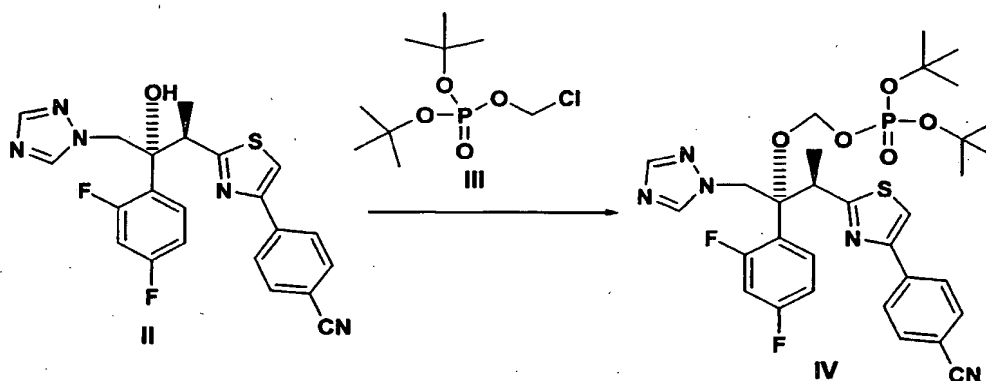
15 (2R,3R)-3-[4-(4-cyanophenyl)thiazol-2-yl]-2-(2,4-difluorophenyl)-1-(1H-1,2,4-triazol-1-yl)-2-[(dihydrogen phosphonoxy)methoxy]butane, sodium salt



20

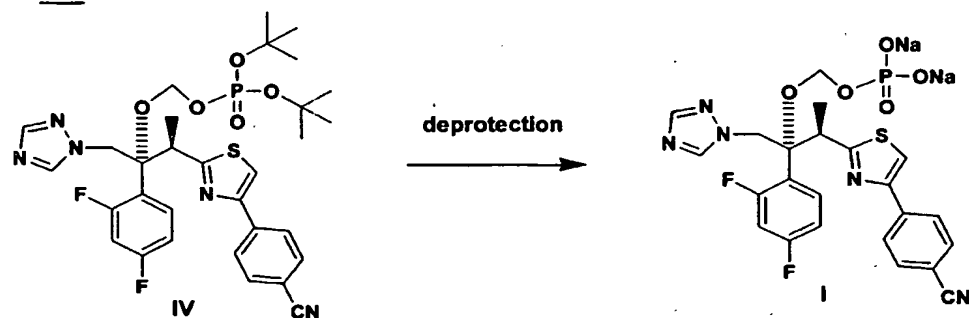
25

A. (2R,3R)-3-[4-(4-cyanophenyl)thiazol-2-yl]-2-(2,4-difluorophenyl)-1-(1H-1,2,4-triazol-1-yl)-2-[(di-tert-butyl phosphonoxy)methoxy]butane



- 5 To a solution of (2R,3R)-3-[4-(4-cyanophenyl)thiazol-2-yl]-2-(2,4-difluorophenyl)-1-(1H-1,2,4-triazol-1-yl)butan-2-ol, II, (8.74 g, 20 mmol) in THF (40 mL) under a nitrogen atmosphere was added sodium hydride (0.80 g, 60% in oil, 20 mmol) at rt. The resulting mixture was stirred at rt for 0.25 h and then di-tert-butyl chloromethyl phosphate, III (10.3 g, 40 mmol) was added. The reaction
- 10 mixture was heated at 50 °C for 16 h. The reaction mixture was then allowed to cool to rt and was concentrated under reduced pressure. The residue was dissolved in Et₂O and was washed with H₂O and brine. The organic layer was dried over MgSO₄ and was concentrated under reduced pressure to obtain 17.0 g of crude subtitled compound, IV, as a gum. A small portion of this crude
- 15 compound was purified by reverse phase chromatography on C-18. The column was eluted with 30% CH₃CN/H₂O, 38% CH₃CN/H₂O, 45% CH₃CN/H₂O and then 50% CH₃CN/H₂O. The product containing fractions were concentrated under reduced pressure in order to remove CH₃CN. The resulting aqueous layer was then extracted with Et₂O. The Et₂O layers were washed with brine, dried and
- 20 concentrated under reduced pressure to afford purified subtitled compound, IV, as a white solid. ¹H NMR (300 MHz, CDCl₃): δ 8.35 (s, 1H), 7.98 (d, 2H, J=9), 7.76 (s, 1H), 7.71 (d, 2H, J=9), 7.63 (s, 1H), 7.36-7.27 (m, 1H), 6.86-6.78 (m, 2H), 5.53 (dd, 1H, J=28,6), 5.53 (dd, 1H, J=9,6), 5.17 (d, 1H, J=15), 5.03 (d, 1H, J=15), 4.01 (q, 1H, J=7), 1.47 (s, 9H), 1.45 (s, 9H), 1.37 (d, 3H, J=7). MS [ESI⁺
- 25 (M+H)⁺] 660.2 obs.

B. (2R,3R)-3-[4-(4-cyanophenyl)thiazol-2-yl]-2-(2,4-difluorophenyl)-1-(1H-1,2,4-triazol-1-yl)-2-[(dihydrogen phosphonoxy)methoxy]butane, sodium salt



5

The crude (2R,3R)-3-[4-(4-cyanophenyl)thiazol-2-yl]-2-(2,4-difluorophenyl)-1-(1H-1,2,4-triazol-1-yl)-2-[(di-tert-butyl phosphonoxy)methoxy]butane, IV, (17 g) was dissolved in CH₂Cl₂ (100 mL). To this solution was added TFA (50 mL) and the reaction mixture was stirred at rt for 0.25 h. The reaction mixture was then concentrated under reduced pressure. To the residue was added H₂O (200 mL), Et₂O (100 mL) and EtOAc (100 mL). The pH of the aqueous layer was adjusted to 7.6 by addition of solid Na₂CO₃ and then the organic and aqueous layers were separated. The aqueous layer was then subjected to reverse phase chromatography on 400 g of C-18 eluted with H₂O to 5% CH₃CN/H₂O. The product containing fractions were concentrated under reduced pressure, frozen and lyophilized to afford 1.5 g of the subtitled compound, I, as a white solid. (1.5 g, 12% over two steps). ¹H NMR (500 MHz, D₂O) δ 8.91 (s, 1H), 7.92 (s, 1H), 7.81 (d, 2H, J=8), 7.80 (s, 1H), 7.77 (d, 2H, J=8), 7.21 (dd, 1H, J=15,9), 6.99 (ddd, 1H, J=9,9,2), 6.91 (ddd, 1H, J=9,9,2), 5.35 (dd, 1H, J=6,6), 5.29 (d, 1H, J=15), 5.21 (dd, 1H, J=6,6), 5.19 (d, 1H, J=15), 3.86 (q, 1H, J=7), and 1.35 (d, 3H, J=7); MS [(ESI⁺ (M-H)⁺ 546.1]; Anal. Calcd for C₂₃H₁₈F₂N₅O₅S₁P₁/Na₂/3.5 H₂O: C, 42.21; H, 3.85; N, 10.70; Na, 7.03. Found: C, 42.32; H, 3.83; N, 10.60; Na, 7.04.

25

Di-tert-butyl chloromethyl phosphate, III:

Di-tert-butyl chloromethyl phosphate, III, may be made by any of the following methods.

5

Method 1

Silver di-t-butyl phosphate (6.34 g, 20 mmol), which was prepared by mixing di-t-butyl phosphate (obtained from di-t-butyl phosphite by the method of Zwierzak and Kluba, Tetrahedron, 1971, 27, 3163) with one equivalent of silver carbonate
10 in 50% aqueous acetonitrile and by lyophilizing to dryness, was placed together with chloriodomethane (35 g, 200 mmol) in benzene and stirred at room temperature for 18 hrs. The reaction mixture was filtered and the filtrate concentrated under reduced pressure. The residue was chromatographed on silica
15 and eluted with 2:1 hexanes-ethyl acetate. Appropriate fractions were concentrated to dryness to obtain the subtitled compound III (3.7 g, 71% yield):
 ^1H NMR (CDCl_3) δ 5.63 (d, 2H, J=17), 1.51 (s, 18H); MS (MH^+ = 259).

Method 2

20

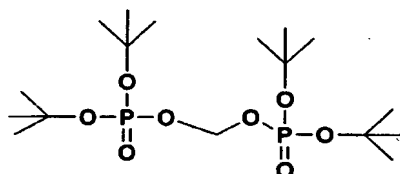
Tetrabutylammonium di-t-butyl phosphate was prepared by dissolving di-t-butyl phosphate [20g, 94 mmol (obtained from di-t-butyl phosphite by the method of Zwierzak and Kluba, Tetrahedron, 1971, 27, 3163)] in methanolic tetrabutylammonium hydroxide (47 mL of 1M solution, 47 mmol). The reaction
25 mixture had a temperature of 23 °C and pH of 4.33. The pH of the reaction mixture was adjusted to 6.5-7.0 by addition of methanolic tetrabutylammonium hydroxide (48 mL of 1M solution, 48 mmol) over 0.2 h. The reaction mixture was stirred for 0.5 h at approximately 26 °C and then was concentrated under reduced pressure at a bath temperature below 40 °C. The crude residue was
30 azeotroped three times by adding toluene (3x100 mL) and then the mixture was concentrated under reduced pressure. The crude residue was then triturated in

cold hexanes (0°C) for 1 h and then the solid was collected by filtration, washed with a minimum amount of cold hexanes and dried to give a first crop of tetrabutylammonium di-*t*-butyl phosphate as a white solid. (24.0g). The mother liquor was concentrated under reduced pressure and then triturated in cold
5 hexanes (20 mL) for 1 h. The solid was collected by filtration, washed with a minimum amount of cold hexanes and dried to give a second crop of tetrabutylammonium di-*t*-butyl phosphate as a white solid. [(8.5g), 32.5g total (77%)]. A solution of tetrabutylammonium di-*t*-butyl phosphate (218 g, 480 mmol) in benzene (200 mL) was added dropwise to stirred chloriodomethane
10 (800g, 4535 mmol) over 1.5 h at rt. The reaction mixture was stirred an additional 1.5 h at rt and then was concentrated under reduced pressure. The oily residue was dissolved in Et₂O and filtered to remove white solids which had precipitated. The organic layer was washed with saturated NaHCO₃ and H₂O/brine (1/1). The organic layer was then dried over magnesium sulfate,
15 filtered and concentrated under reduced pressure to yield a red brown oil (320 g). The red brown oil was subjected to chromatography on silica gel (800g) eluted with 20% EtOAc/Hexanes, 25% EtOAc/Hexanes then 30% EtOAc/Hexanes. The product containing fractions were concentrated under reduced pressure to yield a golden oil. The oil was diluted with CH₂Cl₂ (30 mL), concentrated under
20 reduced pressure and then dried under vacuum to yield the subtitled compound III (61.3g, 49% yield). ¹H NMR (Benzene-d₆) δ 5.20 (2H, d, J=15), 1.22 (18H, s).

Method 3

25 Iodochloromethane (974 g, 402 mL, 5.53 mol) at 25°C was treated with tetrabutylammonium di-*t*-butylphosphate (250 g, 0.553 mol). The phosphate was added portionwise over 10 minutes. The heterogeneous mixture became a clear pink solution after approximately 15 minutes. The mixture was stirred for three hours, and the iodochloromethane was then removed by rotary evaporation with a
30 bath temperature of <30°C. The residue was taken up in 1 L *t*-butyl methyl ether and stirred for 15 minutes to precipitate tetrabutylammonium iodide by-product.

Tetrabutylammonium iodide was removed by vacuum filtration through a sintered glass funnel. The filtrate was concentrated by rotary evaporation to an oil which contained a 5:1 mixture of III and undesired dimer impurity



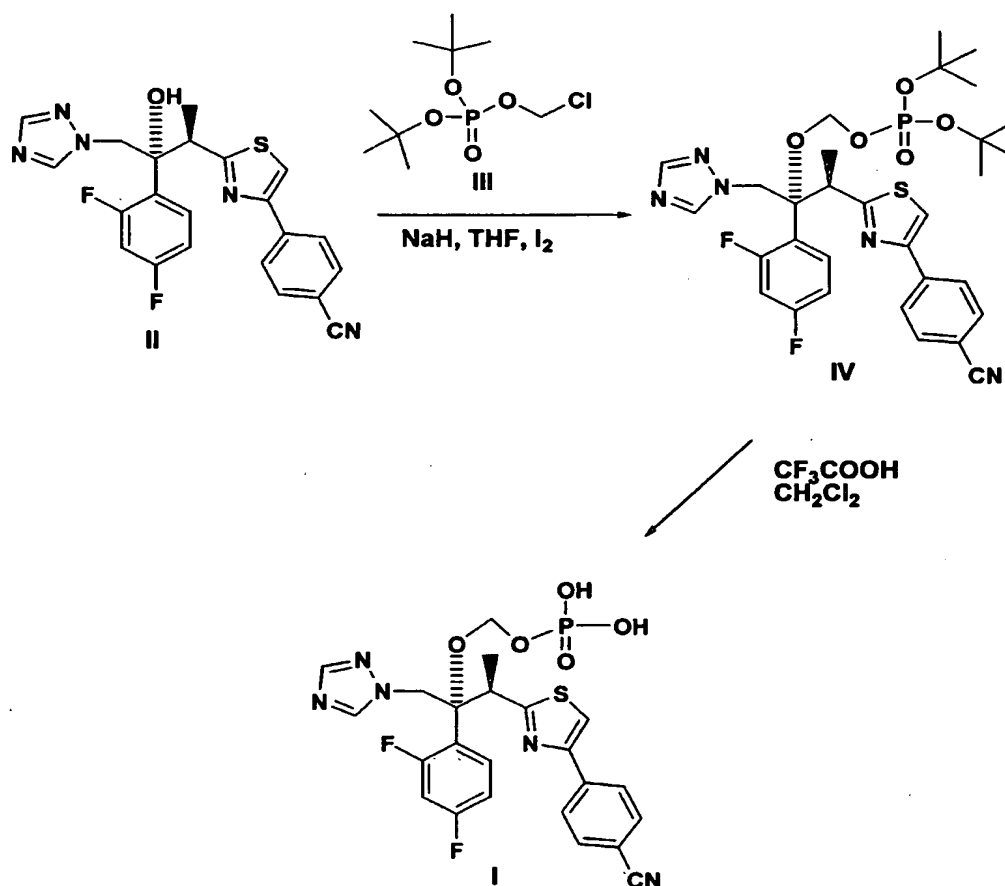
III''

The mixture can be purified by a silica gel chromatography to obtain III as pure compound in ~60% yield as an oil.

EXAMPLE 2

(2R,3R)-3-[4-(4-cyanophenyl)thiazol-2-yl]-2-(2,4-difluorophenyl)-1-(1H-1,2,4-triazol-1-yl)-2-[(dihydrogen phosphonoxy)methoxy]butane

5



- A. An oven dried, 1L round-bottom flask equipped with a mechanical stirrer, nitrogen inlet adapter, pressure-equalizing addition funnel fitted with a rubber septum and temperature probe was charged with sodium hydride (2.89 g, 0.069 mol, 60%) and THF (50 mL). To this stirred suspension, (2R,3R)-3-[4-(4-cyanophenyl)thiazol-2-yl]-2-(2,4-difluorophenyl)-1-(1H-1,2,4-triazol-1-yl)butan-2-ol, II, (10 g, 0.023 mol) in 30 mL of THF was added dropwise over 20 minutes at room temperature. After stirring for 45 minutes, a solution of iodine (2.99 g,

0.0115 mol) in THF (30 mL)) was added dropwise over 10 minutes followed by dropwise addition of compound di *tert* butylchloromethyl phosphate, III (13.29 g, 0.035 mol, ~68% purity) over 15 minutes. The reaction mixture was stirred for 4 hours at about 41 °C to complete the reaction. The completion of the reaction
5 was judged by in-process HPLC. The reaction mixture was poured into ice cold water (100 mL). The aqueous phase was separated and extracted with ethyl acetate (3 x 50 mL) and the combined organic extract was washed with 10% sodium thiosulfite (50 mL), water (50 mL), brine (50 mL), dried over magnesium sulfate and concentrated under reduced pressure to give pale yellow oil (22.8 g,
10 In-process HPLC: ~ 97% pure). The crude product was used "as is" in step B.

B. To a round-bottom flask equipped with magnetic stirrer, cooling bath, pH probe and N₂ inlet-outlet was charged the product of Step A above (7.5 g) in CH₂Cl₂ (23 mL) and cooled to 0 °C. To this stirred solution, trifluoroacetic acid
15 (8.8 mL) was added slowly and stirred for 3 h to complete the reaction. The completion of the reaction was judged by in-process HPLC. The reaction mixture was poured into a cold solution of 2N NaOH (64 mL). The reaction mixture was extracted with t-butyl acetate (2 x 65 mL) to remove all the organic impurities. The aqueous layer containing the title product as bis sodium salt was treated with
20 activated charcoal (10 g) and filtered through a bed of Celite. The clear filtrate was acidified with 1N HCl to pH 2.5. The free acid, the title product, was extracted into ethyl acetate (2 x 50 mL). The combined organic layer was washed with water, dried over MgSO₄, filtered, and the filtrate concentrated under reduced pressure to afford 3.39 g of crude title product.

25

30

EXAMPLE 3

5 Bis lysine salt of (2R,3R)-3-[4-(4-cyanophenyl)thiazol-2-yl]-2-(2,4-
difluorophenyl)-1-(1H-1,2,4-triazol-1-yl)-2-[(dihydrogen
phosphonoxy)methoxy]butane

The above obtained title product from Example 2 was dissolved in methanol (75 mL) and to this L-lysine (1.8 g) was added and heated at 60 °C for 4.5 h. The hot reaction mixture was filtered through a bed of Celite. The filtrate
10 was concentrated to about 5 mL, mixed with ethanol (100 mL) and heated to 65 °C to crystallize the bis lysine salt. The salt was collected on a Buchner funnel and dried under vacuum to afford 3.71 g of the title compound as an off white crystalline solid.

15

EXAMPLE 4

Tert-butyl amine salt of (2R,3R)-3-[4-(4-cyanophenyl)thiazol-2-yl]-2-(2,4-
difluorophenyl)-1-(1H-1,2,4-triazol-1-yl)-2-[(dihydrogen
phosphonoxy)methoxy]butane

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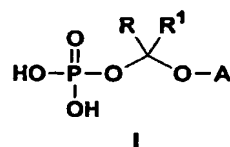
A solution of title product of Example 2 was dissolved in 50 mL of ethyl acetate and to this was added t-butyl amine (5.3 mL) under nitrogen. The reaction mixture was stirred at 40 °C for about 1 hour to crystallize the product. The bis t-butyl amine salt was collected on a Buchner funnel and dried under vacuum to
25 afford 2.21 g of the title compound as an off white crystalline solid.

CLAIMS

We claim:

1. A compound of the formula

5

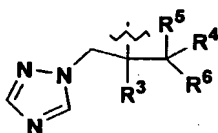


wherein A is the non-hydroxy portion of a triazole antifungal compound of the type containing a secondary or tertiary hydroxy group, and R and R¹ are each independently hydrogen or (C₁-C₆)alkyl, or a pharmaceutically acceptable salt thereof.

10

2. A compound of claim 1 wherein A represents the non-hydroxy portion of a triazole antifungal compound of the type containing a tertiary hydroxy group.

- 15 3. A compound of claim 2 wherein A is a group of the formula



wherein R³ represents a phenyl group substituted by one or more halogen atoms;

R⁴ represents hydrogen or CH₃;

20

R⁵ represents hydrogen, or taken together with R⁴ may represent =CH₂;

R⁶ represents a 5- or 6-membered nitrogen-containing ring which may be optionally substituted by one or more groups selected from halogen, =O,

phenyl substituted by one or more groups selected from CN, (C₆H₄)-OCH₂CF₂CHF₂ and CH=CH-(C₆H₄)-OCH₂CF₂CHF₂, or phenyl substituted by one or more groups selected from halogen and methylpyrazolyl.

5

4. A compound as claimed in claim 3 wherein R³ is 2,4-difluorophenyl.

5. A compound as claimed in claim 4 wherein R⁴ is methyl and R⁵ is hydrogen.

10

6. A compound as claimed in claim 5 wherein R⁶ is 4-(4-cyanophenyl)-thiazol-2-yl.

15

7. A compound of claim 6 wherein R and R¹ are each hydrogen, or a pharmaceutically acceptable salt thereof.

20

8. The compound named (2R,3R)-3-[4-(4-cyanophenyl)thiazol-2-yl]-2-(2,4-difluorophenyl)-1-(1H-1,2,4-triazol-1-yl)-2-[(dihydrogen phosphonoxy)methoxy]butane, or a pharmaceutically acceptable salt thereof.

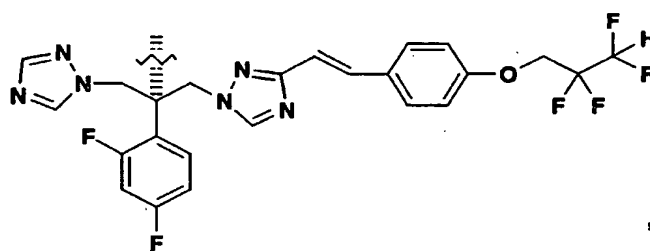
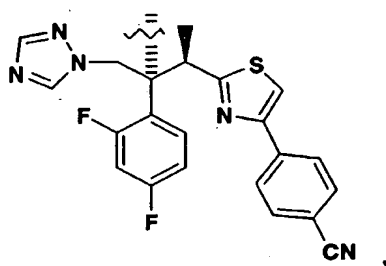
9. The crystalline bis lysine salt of (2R,3R)-3-[4-(4-cyanophenyl)thiazol-2-yl]-2-(2,4-difluorophenyl)-1-(1H-1,2,4-triazol-1-yl)-2-[(dihydrogen phenoxy)methoxy]butane.

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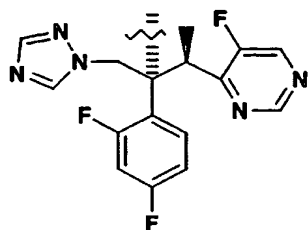
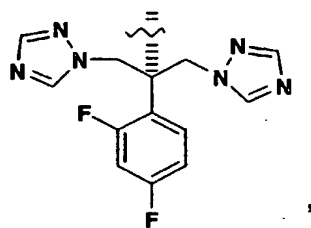
10. The crystalline tertiary-butylamine salt of (2R,3R)-3-[4-(4-cyanophenyl)thiazol-2-yl]-2-(2,4-difluorophenyl)-1-(1H-1,2,4-triazol-1-yl)-2-[(dihydrogen phenoxy)methoxy]butane.

11. A compound of claim 1 where A is

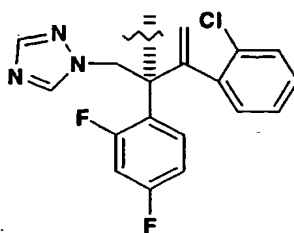
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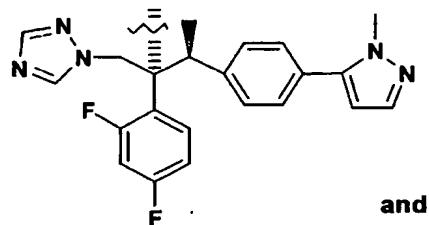
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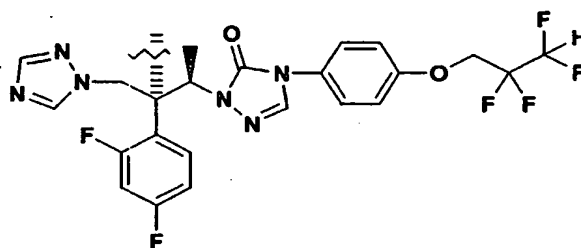
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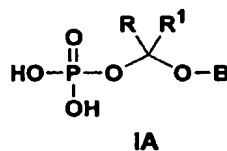


and



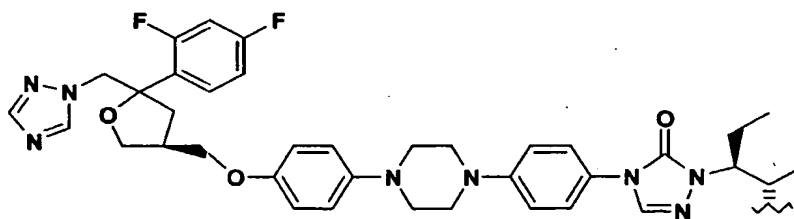
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12. A compound of the formula



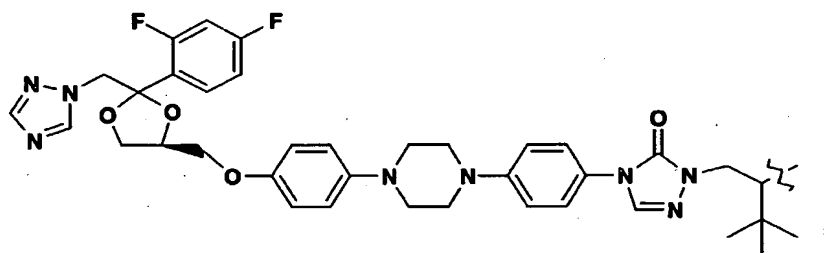
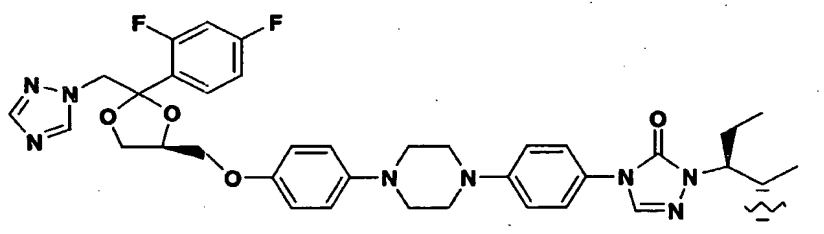
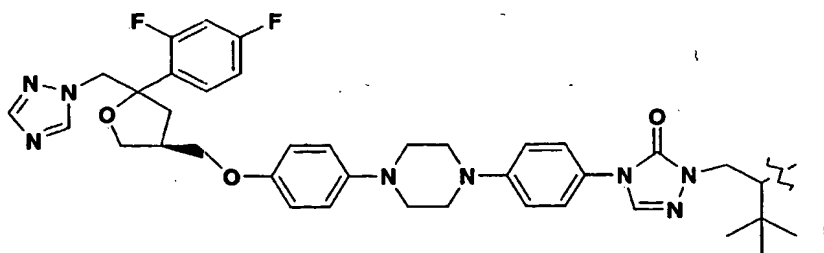
wherein B is the non-hydroxy portion of a triazole antifungal compound of the
 10 type containing a secondary hydroxy group, and R and R¹ can each independently
 be hydrogen or (C₁-C₆)alkyl, or a pharmaceutically acceptable salt thereof.

13. A compound of claim 12 wherein B is either:

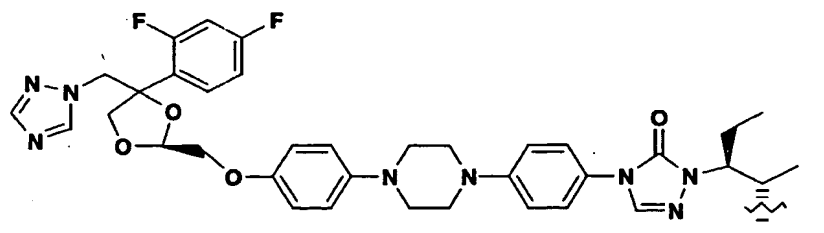


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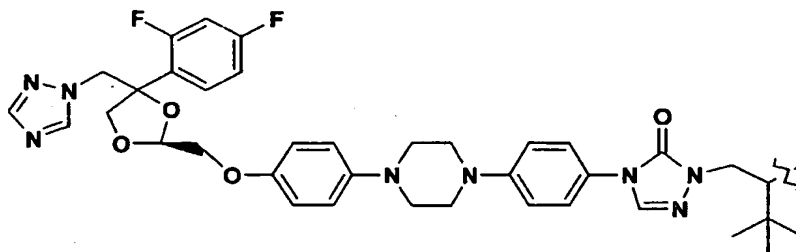


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or

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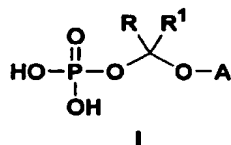


14. A method for the treatment of fungal infections, which comprises administering an effective antifungal amount of a compound of claim 1, or a pharmaceutically acceptable salt thereof, to a mammalian host in need thereof.

15. A pharmaceutical composition comprising a compound of claim 1, or a pharmaceutically acceptable salt thereof, in admixture with a pharmaceutically acceptable adjuvant, diluent, or carrier.

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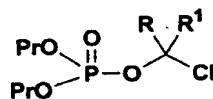
16. A process for the preparation of a water-soluble prodrug of the formula



15 wherein A is the non-hydroxy portion of a triazole antifungal compound of the type containing a secondary or tertiary hydroxy group, R and R¹ are each independently hydrogen or (C₁-C₆)alkyl, or a pharmaceutically acceptable salt thereof, which comprises

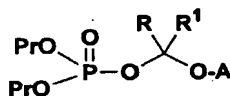
20 (a) reacting a compound of the formula A-OH wherein A is the non-hydroxy portion of a triazole antifungal compound of the type containing a secondary or tertiary hydroxy group with a compound of the formula

33



III

in which R and R¹ are as defined above and Pr represents a hydroxyl-protecting group in an inert organic solvent in the presence of base at a temperature of from about 25°C to 50°C to form an intermediate of the formula

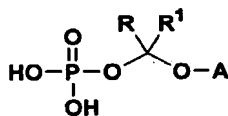


IV

wherein Pr, A, R and R¹ are as defined above, and

10

(b) removing the protecting groups Pr by conventional means to produce a compound of the formula



I

15

and, if desired, converting said compound I by conventional means to a pharmaceutically acceptable salt thereof.

17. The process of claim 16 wherein the protecting group Pr is tertiary-butyl.

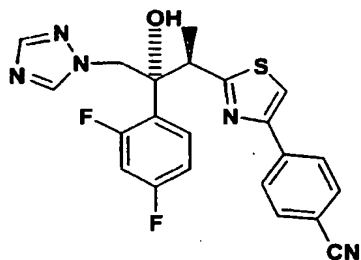
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18. The process of claim 16 wherein the solvent used in Step (a) is tetrahydrofuran.

19. The process of claim 16 wherein the base used in Step (a) is sodium hydride.

20. The process of claim 16 wherein starting material A-OH is

5



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/01284

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 31/496; C07F 9/06

US CL : 514/254.01, 254.07, 254.1; 544/337; 548/119

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/254.01, 254.07, 254.1; 544/337; 548/119

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAS-ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	WO 00/30655 A1 (BRISTOL-MYERS SQUIBB COMPANY) 02 June 2000 (02.06.00).	1-20

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

10 MAY 2001

Date of mailing of the international search report

06 JUN 2001

Name and mailing address of the ISA/US
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TAOFIQ A. SOLOLA

Telephone No. (703) 308-1235

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/01284

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/01284

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1-11, 14-20, drawn to compounds of formula I, their composition, method of use and process of making. Group II, claim(s) 12-13, drawn to compounds of formula IA.

The inventions listed as Groups I-II do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the same or corresponding technical feature among the groups is phosphorous acid ester which is well known in the art. Therefore under PCT Rules 13.1 and PCT Rule 13.2, phosphorous acid ester does not constitute a special technical feature.

The corresponding technical feature among the compounds of group I is formula I. The corresponding technical feature among the compounds of group II is formula IA.